



Motor unit number index and compound muscle action potential amplitude

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HIGHLIGHTS

- MUNIX is almost entirely dependent on CMAP amplitude in healthy controls and patients.
- MUNIX is particularly misleading for muscles with normal or small MUs, due to superimposition of MUPs.
- MUNIX should not be used as a measure of the number of functional motor units in a muscle.

ABSTRACT

Objectives: MUNIX (motor unit number index), derived from the compound muscle action potential (CMAP) and surface EMG interference pattern (SIP) has become popular as a substitute for motor unit number estimation (MUNE). This study was undertaken to determine why, in recent recordings from amyotrophic lateral sclerosis (ALS) patients and healthy controls, we found that MUNIX values resembled CMAP amplitudes more closely than MUNE values.

Methods: The relationship between MUNIX and CMAP and SIP amplitudes was investigated by a theoretical analysis and by reanalysing the data from the previous study.

Results: Theory indicates that when motor unit potentials overlap extensively, information about motor unit size and number is lost, and MUNIX depends only on CMAP area and power. Accordingly, MUNIX values were found to be sensitive to changes in CMAP amplitude but insensitive to changes in SIP amplitude. The reproducibility of MUNIX measurements in healthy controls was found to depend almost entirely on correlation with CMAP properties.

Conclusions: MUNIX gives misleading information about motor unit numbers in healthy controls, and provides little information about loss of motor units in ALS patients beyond that given by simple CMAP amplitude measurements.

Significance: MUNIX should not be interpreted as a MUNE method.

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1. Introduction

Motor unit number index (MUNIX) was proposed by Nandedkar and colleagues as a quick method to estimate the number of functional units in a muscle, by comparing the area and power of the compound muscle action potential (CMAP) with that of the surface EMG interference pattern (SIP) (Nandedkar et al., 2004). Although it was not described as a motor unit number estimation (MUNE) method,

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MUNIX was intended to serve the same purpose as MUNE, and was scaled 'to give measurements similar to the MU number estimates on other techniques' (Nandedkar et al., 2004). A motor unit size index (MUSIX) was also proposed, by dividing the CMAP amplitude by MUNIX. This method has been incorporated into the software of commercial EMG machines and has proved increasingly popular as a means to assess neuromuscular function, since it is much quicker and easier than traditional methods of MUNE (Neuwirth et al., 2018). Recently another quick MUNE method was proposed: MScan-Fit, in which a detailed stimulus-response function or CMAP scan (Maathuis et al., 2011) is fitted by a model (Bostock, 2016).

We have recently undertaken a detailed comparison of these two methods with a more traditional MUNE method, multiple point stimulation MUNE (MPS) (Doherty and Brown, 1993). The first paper explored intra- and inter-operator variability in 20 normal subjects and 22 ALS patients (Jacobsen et al., 2017) while the second followed changes in the patients over 4 and 8 months (Jacobsen et al., 2019). In the first paper we found a higher correlation between log MPS MUNE and log MScan MUNE for all 42 subjects ($R^2 = 0.962$) than between either log MUNE method and log MUNIX ($R^2 = 0.832, 0.891$). On the other hand, quadratic regression showed a higher correlation between CMAP amplitude and MUNIX ($R^2 = 0.944$), than between CMAP amplitude and either MUNE method ($R^2 = 0.724, 0.801$). In Table 3 of the second paper we published figures for Spearman's rank correlation coefficient for all 66 ALS recordings, and again found higher correlations between the two MUNE methods ($\rho = 0.918$) and between MUNIX and CMAP amplitude ($\rho = 0.919$) than between MUNIX and the MUNE methods ($\rho = 0.850, 0.868$). The MUNE methods also correlated better with the revised ALS functional rating score (ALSFRS-R) ($\rho = 0.597, 0.568$) than did either MUNIX ($\rho = 0.451$) or CMAP amplitude ($\rho = 0.452$). In both studies, therefore, we found that the two MUNE methods, although quite different, gave very similar results, whereas MUNIX values were more closely related to CMAP amplitude.

The present study was undertaken to better understand how MUNIX was related to CMAP amplitude. First, the MUNIX analysis was reformulated in terms of the SIP form factor, where form factor (G) is the ratio between Root-Mean-Square (RMS) and mean rectified value of a continuous waveform. This helped to make it clear why MUNIX values are so strongly dependent on CMAP amplitude. Secondly, the recordings of CMAPs and voluntary EMG signals in the previous study were reanalysed to show the separate effects of changing the sizes of CMAPs or SIPs only, and finally, the claim that MUNIX shows good inter- and intra-rater reliability in healthy subjects (Neuwirth et al., 2011) was assessed by testing inter- and intra-rater correlations for dependence on CMAP amplitude.

1.1. Theory

MUNIX is an EMG measure derived from the compound muscle action potential (CMAP) and the surface EMG interference pattern (SIP) that is intended to provide an index of motor unit numbers. Nandedkar and colleagues defined the 'ideal case motor unit count' (ICMUC) by the formula:

$$\text{ICMUC} = \frac{[\text{CMAP power}] \times [\text{SIP area}]}{[\text{CMAP area}] \times [\text{SIP power}]} \quad (1)$$

The SIP is a continuous function of time, so that area and power are defined for a particular length of time, e.g. 300 ms, as in this study and in Nandedkar et al., 2010.

$$\text{i.e. } [\text{SIP area}_{300}] = \int |y| \cdot dt \text{ from } t = 0 \text{ to } 300 \quad (\text{units : mV} \cdot \text{ms}) \quad (2)$$

$$[\text{SIP power}_{300}] = \int y^2 \cdot dt \text{ from } t = 0 \text{ to } 300 \quad (\text{units : mV}^2 \cdot \text{ms}) \quad (3)$$

With continuous functions of time, it is often more convenient to measure the mean (i.e. mean absolute value) and RMS values. We can therefore write:

$$[\text{SIP mean}] = \int |y| \cdot dt / \int dt \text{ from } t = 0 \text{ to } 300 \\ = [\text{SIP area}_{300}] / 300 \quad (\text{units : mV}) \quad (4)$$

$$[\text{SIP RMS}] = \sqrt{(\int y^2 \cdot dt / \int dt) \text{ from } t = 0 \text{ to } 300} \\ = \sqrt{([\text{SIP power}_{300}] / 300)} \quad (\text{units : mV}) \quad (5)$$

$$\text{Then ICMUC} = \frac{[\text{CMAP power}] \times [\text{SIP mean}]}{[\text{CMAP area}] \times [\text{SIP RMS}]^2} \quad (6)$$

In electronics the ratio RMS/mean of a continuous waveform is known as the form factor (G), which depends on waveform but not on amplitude or frequency. The form factor varies over a limited range for different waveforms. Thus for square waves $G = 1$, for sine waves $G = \pi/(2\sqrt{2})$ (~ 1.112), and for Gaussian noise $G = \sqrt{(\pi/2)}$ (~ 1.253). The form factor does, however, increase when there are gaps in a waveform, so that whereas G for a continuous sine-wave is $\pi/(2\sqrt{2})$, if single cycles are separated by gaps of length n cycles, G is increased by a factor of $\sqrt{(n+1)}$. G is also increased by unevenness in a continuous waveform, so that if 1 in n cycles is increased by a factor m , then G is increased by the factor $\sqrt{n \times \sqrt{(m^2 + n - 1)} / (m + n - 1)}$.

Replacing RMS/mean by G , the expression for ICMUC can be written:

$$\text{ICMUC} = \frac{[\text{CMAP power}]}{[\text{CMAP area}]} \times \frac{1}{[G_{\text{SIP}}]^2} \times \frac{1}{[\text{SIP mean}]} \quad (7)$$

From the relationship between ICMUC and [SIP.Area], MUNIX is arbitrarily defined as the value of ICMUC when [SIP.Area] for a 1 sec epoch is 20 mV.ms, i.e. when [SIP mean] = 0.02 mV = 20 μ V (Nandedkar et al., 2010). Inserting these values gives us an expression for MUNIX in terms of G_{SIP} :

$$\text{MUNIX} = \frac{[\text{CMAP power}]}{[\text{CMAP area}]} \times \frac{50}{[G_{\text{SIP}(20\mu\text{V})}]^2} \quad (8)$$

where $G_{\text{SIP}(20\mu\text{V})}$ is the value of G_{SIP} when [SIP mean] = 20 μ V. MUNIX can therefore only estimate the number of motor units if $G_{\text{SIP}(20\mu\text{V})}$ provides information about the motor unit amplitudes, otherwise MUNIX is determined solely by the CMAP.

2. Methods

The methods involved in the studies on healthy controls subjects and ALS patients are fully described in the previous paper (Jacobsen et al., 2017). Twenty-one patients with ALS or progressive muscular atrophy (PMA) (6 females and 15 males, aged 47–83, mean 66.3 years) and 20 healthy age- and sex-matched healthy subjects (7 females and 13 males, aged 44–76, mean 65.6 years) were studied between December 2015 and June 2016. (N.B. There were actually 22 patients in the original study, but MUNIX could not be calculated in one, because of low CMAP amplitude, so only 21 are included in this reanalysis.)

MUNIX recordings were made with a Keypoint version 2.11 (Dantec, Skovlunde, Denmark), stimulating at a frequency of 1 Hz and using a 300 ms window. CMAPs and a series of 10 SIPs with increasing levels of voluntary contraction were recorded from abductor pollicis brevis (APB) as previously described in detail (Jacobsen et al., 2017). The recordings were exported to a Microsoft Excel file for the MUNIX analysis (Neuwirth et al., 2015). The values exported to Excel were CMAP amplitude, power and area and 10 SIP powers and areas over 300 ms. The mean SIP value in mV was calculated by dividing the SIP area in mV.ms by the 300 ms recording period (see Eq. (4)), and SIP RMS values by taking the square root of the SIP power in mV².ms divided by 300 (Eq. (5)).

To evaluate the separate effects of changes by a factor F in (a) CMAP amplitude and (b) the surface motor unit potentials making up the SIPs, the values exported to Excel were modified as follows: (a) CMAP amplitude and area were multiplied by F and CMAP power by F^2 ; (b) the 10 SIP areas were multiplied by F and SIP pow-

ers were multiplied by F^2 . For each patient and control subject, the estimates of MUNIX and MUSIX were evaluated (a) for halving and doubling CMAP amplitudes (with SIPs unchanged), and (b) for halving and doubling SIP amplitudes (with CMAPs unchanged).

To assess the degree to which the reproducibility of MUNIX values is due to their dependence on CMAP amplitude, correlations between the repeated recordings on the same healthy subjects by both the same and different operators were compared with partial correlations, after allowing for correlation with CMAP amplitude.

All MUNIX and MUSIX values were derived by the MUNIX Excel program supplied as part of the Keypoint.net software (www.neurolite.ch). The plots and linear correlations were carried out by QtracP software (© Institute of Neurology, University College, London, distributed by Digitimer Ltd at www.digitimer.com), and the partial correlations by MedCalc (www.medcalc.org). P values <0.05 were considered significant.

3. Results

3.1. Relationship between SIP.area and SIP.RMS

Fig. 1A shows the relationship between [SIP mean] and [SIP RMS] for the 200 SIP epochs recorded from healthy control subjects, and Fig. 1B the same relationship for the 210 SIP epochs recorded from patients. In each case the points fall close to the straight line relationship:

$$[\text{SIP RMS}] = \sqrt{(\pi/2)} \times [\text{SIP mean}] \quad (9)$$

or $G_{\text{SIP}} = 1.253$, which is the value for a Gaussian distribution of amplitudes (as might be expected by the central limit theorem for the summation of many independent units). However, although it is clear from Fig. 1A that G_{SIP} approaches $\sqrt{(\pi/2)}$ as [SIP mean] becomes large, MUNIX depends on $G_{\text{SIP}(20\mu\text{V})}$ (Eq. (8)), which cannot readily be resolved in Fig. 1. The data in Fig. 1A are therefore replotted in Fig. 2A on log-log coordinates. The points are again fitted very well by a straight line, but the slope is slightly less than 1, so that G increases as the SIPs decrease. The regression line cuts the vertical dashed line where [SIP mean] = 0.02 mV at [SIP RMS] = 0.0282, giving a mean $G_{\text{SIP}(20\mu\text{V})}$ value of 1.421. The actual values of $G_{\text{SIP}(20\mu\text{V})}$ for the 20 healthy control subjects are plotted in Fig. 2C as a function of CMAP amplitude, and it can be seen that although there is a trend for $G_{\text{SIP}(20\mu\text{V})}$ to be higher for smaller CMAPs, the points all

fall close to the value of 1.421 given by the dashed line. We can therefore write for the healthy controls:

$$\text{MUNIX} \approx \frac{[\text{CMAP power}]}{[\text{CMAP area}]} \times \frac{50}{1.421^2}$$

or:

$$\text{MUNIX} \approx \frac{[\text{CMAP power}]}{[\text{CMAP area}]} \times 24.76 \quad (10)$$

The ratio [CMAP power]/[CMAP area] is closely related to [CMAP amplitude], as shown in Fig. 3A. From the slope, we can therefore write for the healthy controls:

$$\text{MUNIX} \approx [\text{CMAP amplitude}] \times 0.809 \times 24.76$$

or:

$$\text{MUNIX} \approx [\text{CMAP amplitude}] \times 20.0 \quad (11)$$

and, since MUSIX is defined as [CMAP amplitude]/MUNIX:

$$\text{MUSIX} \approx 1/20.0 \text{ mV} \approx 50 \mu\text{V} \quad (12)$$

This explains why the MUSIX values of the healthy controls cluster about 50 μV (median = 49.5 μV) (see below) and why these values are unaffected by changes in SIP amplitude.

For the patients, the corresponding plots are shown in Figs. 2B, D and 3B. Although the regression line in Fig. 2B is similar to that in Fig. 2A, there is more scatter about the line, and there is much more variability in values of $G_{\text{SIP}(20\mu\text{V})}$, with a marked increase above the 1.421 line for some patients with small CMAPs.

3.2. Dependence of MUNIX and MUSIX on CMAP and SIP amplitudes

The changes in MUNIX for the 20 healthy control subjects and 21 patients produced by halving and doubling CMAP amplitudes are illustrated in Fig. 4A, and the changes produced by halving and doubling motor unit potentials are illustrated in Fig. 4B. The corresponding changes in MUSIX are illustrated in Fig. 5A and B. The mean changes, expressed as factors, are listed in Table 1. It can be seen that, as expected, changing CMAP amplitudes by a factor F , while leaving motor unit potentials unchanged, changes MUNIX by the same factor F (Fig. 3A) while leaving MUSIX unchanged (Fig. 4A). On the other hand, changing motor unit potentials by a factor of F does not have the expected effect on either MUNIX (Fig. 3B) or MUSIX (Fig. 4B). For example, doubling motor unit amplitudes, while keeping the CMAP amplitudes unchanged (i.e. $\text{SIP} \times 2$,

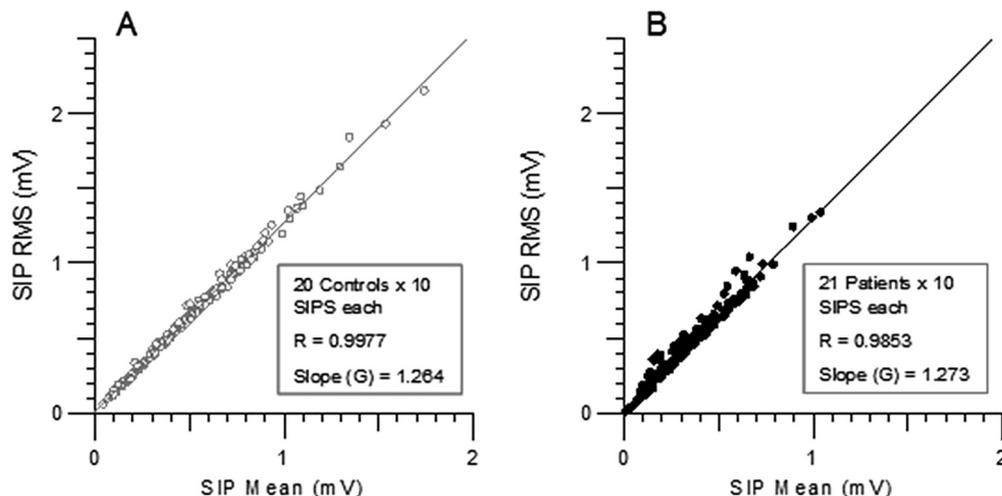


Fig. 1. The relationship between RMS and mean rectified values of the surface EMG interference pattern (SIP) for 10 300 ms epochs recorded from 20 healthy control subjects (A) and for 21 patients (B). The ratio RMS/mean or form factor G is similar in each case to that expected for Gaussian noise.

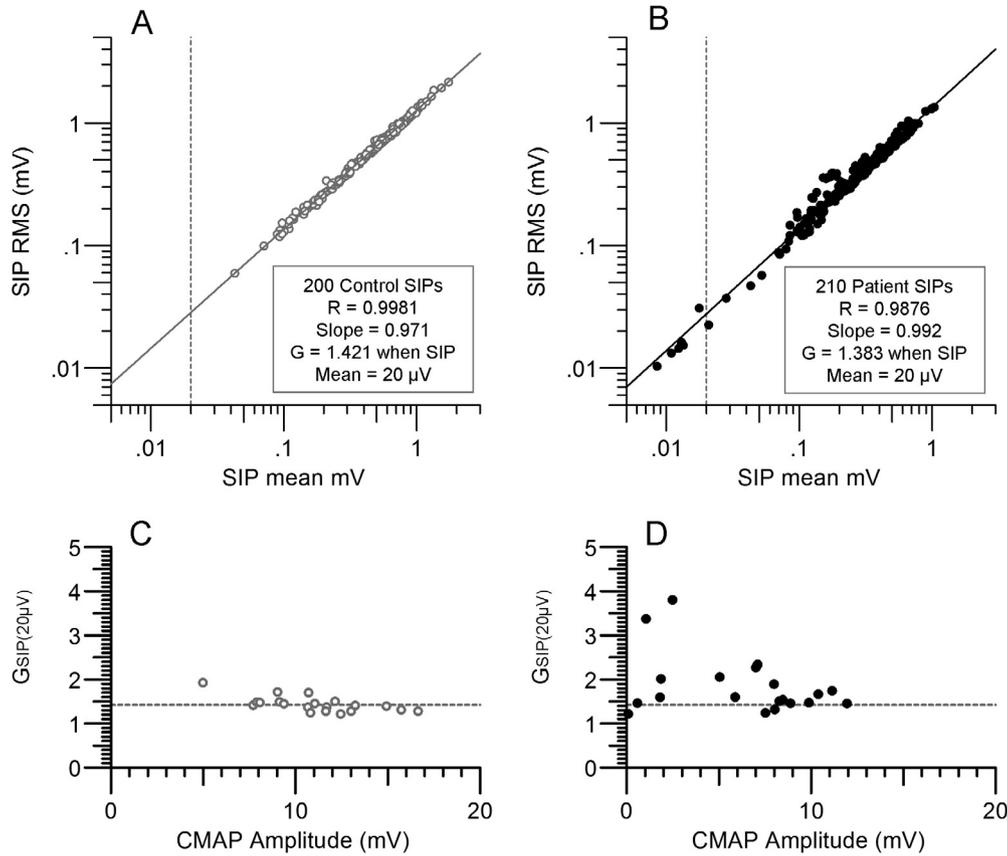


Fig. 2. Form factor $G_{\text{SIP}(20\mu\text{V})}$ for group data and individual subjects. The log–log plots for healthy controls (A) and for patients (B) (same data as Fig. 1) show that the mean form factor increases slightly at low levels of activity, so that $G_{\text{SIP}(20\mu\text{V})}$ is greater than the value of G for Gaussian noise. The values of $G_{\text{SIP}(20\mu\text{V})}$ for individual subjects are illustrated below for healthy controls (C) and for patients (D), compared with the mean value for the controls of 1.421, indicated by the dashed lines.

CMAP $\times 1$) should double the estimated motor unit size index MUSIX, but only increases it by a factor of 1.044 in controls and by an average of 1.183 in patients (Table 1).

3.3. Dependence of intra-rater and inter-rater reproducibility on CMAP amplitude

In our previous paper, each MUNIX measurement was performed twice by two operators, to allow testing of intra- and inter-operator reproducibility. However, we did not test whether

the apparent reproducibility of the MUNIX measurements was simply a reflection of the reproducibility of the CMAP measurements. The results of such an assessment are shown in Table 2. Correlations between two MUNIX measurements averaged 0.787 for intra-operator comparisons and 0.785 for inter-operator comparisons (Table 2A), whereas those for CMAP amplitudes were somewhat higher, at 0.849 for intra-operator and 0.864 for inter-operator comparisons (Table 2B). These values are similar to the ICCs previously given for a total of 230 measurements on 5 muscles, i.e. 0.726 for MUNIX and 0.840 for CMAP amplitude

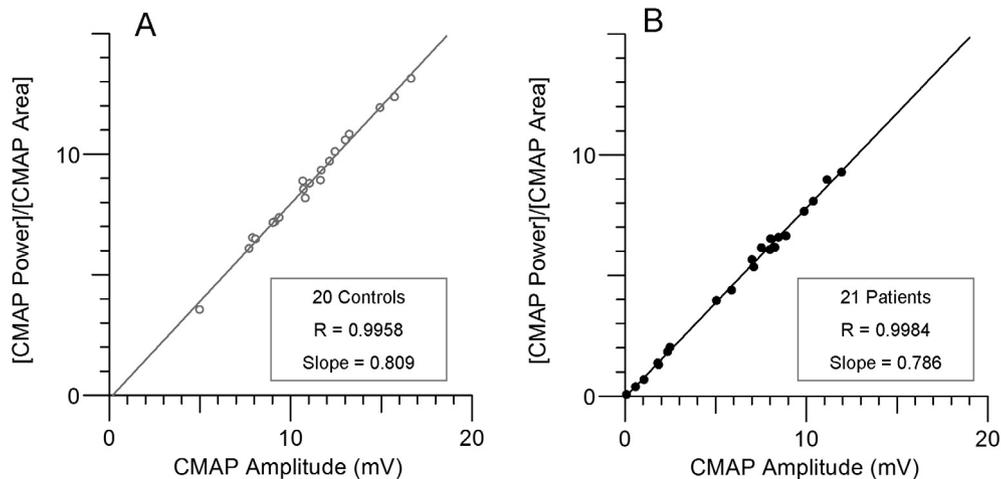


Fig. 3. Relationship between the ratio [CMAP power]/[CMAP area], as measured by the MUNIX software, and CMAP amplitude for healthy control subjects (A) and for patients (B). The relationships are very similar, with [CMAP power]/[CMAP area] in each case close to $0.8 \times$ [CMAP amplitude].

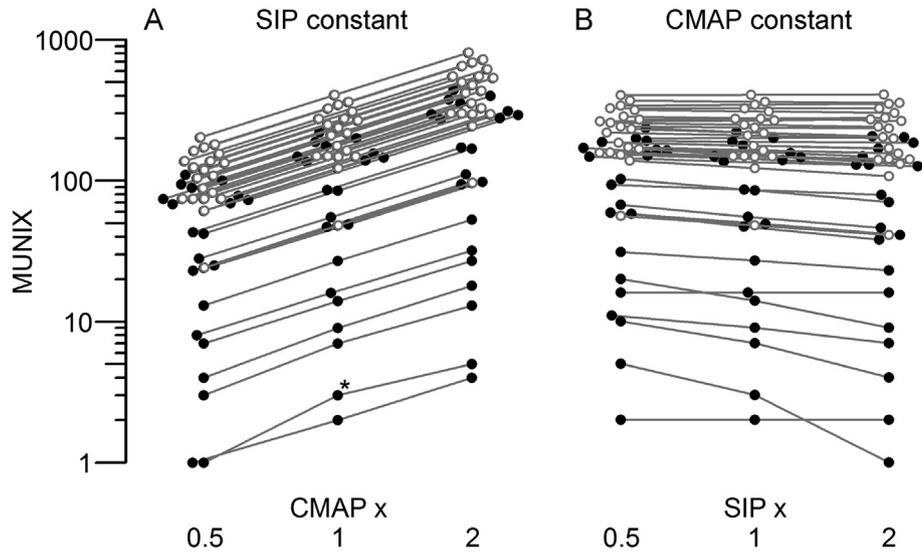


Fig. 4. Changes in MUNIX values of 20 healthy control subjects (grey open circles) and 21 patients (black filled circles) when (A) CMAP amplitudes are multiplied by 0.5 or 2 while keeping SIP amplitudes constant, and (B) when SIP amplitudes are changed and CMAP amplitudes are kept constant. The changes in (A) are as expected for the number of motor units, but MUNIX was much less sensitive to changes in motor unit amplitude, especially for control subjects, than a true MUNE would be. The lines join measurements derived from the same subject. (N.B. The points for the subject marked by the * in (A) do not fall on the same straight line as for the other subjects simply because MUNIX values are registered to the nearest integer.)

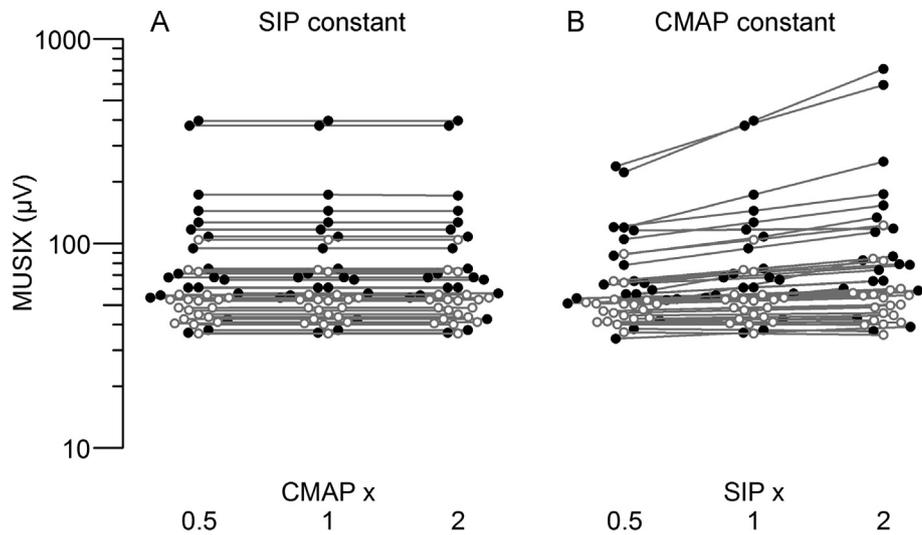


Fig. 5. Changes in MUSIX (motor unit size index) values of 20 healthy control subjects (grey open circles) and 21 patients (filled black circles) when (A) CMAP amplitudes are multiplied by 0.5 or 2 while keeping SIP amplitudes constant, and (B) when SIP amplitudes are changed and CMAP amplitudes are kept constant. The changes in (A) are as expected for motor unit sizes, but in (B) MUSIX did not behave like motor unit sizes when SIP amplitudes were changed, especially for the control subjects.

Table 1

Mean changes in MUNIX and MUSIX with changes in CMAP and SIP amplitudes, compared with changes expected for MUNE and motor unit potential (MUP) size.

	SIP × 1 CMAP × 0.5	SIP × 1 CMAP × 2	SIP × 0.5 CMAP × 1	SIP × 2 CMAP × 1
MUNE change expected	×0.5	×2	×2	×0.5
MUNIX change found in healthy control subjects	×0.5	×2	×1.044	×0.960
MUNIX change found in patients	×0.485*	×1.973 [†]	×1.164	×0.850
MUP change expected	×1	×1	×0.5	×2
MUSIX change found in healthy control subjects	×1	×1	×0.960	×1.044
MUSIX change found in patients	×1	×1	×0.864	×1.183

* Indicates values that are not quite as expected because MUNIX values are expressed to the nearest integer.

(Neuwirth et al., 2011). Although Neuwirth and colleagues noted in that study that MUNIX was highly correlated with CMAP amplitude, they did not test whether the MUNIX reproducibility might

be dependent on the CMAP reproducibility. This is done in Table 2C, where the partial correlations show that the MUNIX correlations become almost entirely non-significant when

Table 2

Intra- and inter-operator correlations for recordings on 20 normal subjects. A: Linear correlation coefficients between MUNIX values recorded in two separate sessions by operators HT and ABJ. B: Correlations between CMAP amplitudes recorded in the same sessions as in A, C: Partial correlations between MUNIX values, as in A, after allowing for covariance with CMAP amplitudes.

	HT-1	HT-2	ABJ-1	ABJ-2
<i>A: Correlations between MUNIX values</i>				
HT-1	–	0.840 ^{****}	0.768 ^{***}	0.768 ^{***}
HT-2	0.840 ^{****}	–	0.779 ^{***}	0.826 ^{****}
ABJ-1	0.768 ^{***}	0.779 ^{***}	–	0.734 ^{***}
ABJ-2	0.768 ^{***}	0.826 ^{****}	0.734 ^{***}	–
<i>B: Correlations between CMAP amplitudes</i>				
HT-1	–	0.904 ^{****}	0.841 ^{****}	0.849 ^{****}
HT-2	0.904 ^{****}	–	0.861 ^{****}	0.904 ^{****}
ABJ-1	0.841 ^{****}	0.861 ^{****}	–	0.794 ^{****}
ABJ-2	0.849 ^{****}	0.904 ^{****}	0.794 ^{****}	–
<i>C: Partial correlations between MUNIX values, allowing for covariance with CMAP amplitudes</i>				
HT-1	–	0.359 ^{NS}	0.284 ^{NS}	0.023 ^{NS}
HT-2	0.359 ^{NS}	–	0.476 [*]	0.305 ^{NS}
ABJ-1	0.284 ^{NS}	0.476 [*]	–	–0.024 ^{NS}
ABJ-2	0.023 ^{NS}	0.305 ^{NS}	–0.024 ^{NS}	–

Superscripts indicate probabilities that correlations were due to chance:

- ^{NS} P > 0.05.
^{*} P < 0.05.
^{***} P < 0.001.
^{****} P < 0.0001.

dependence on CMAP amplitude is taken into account. (The one exception, ABJ-1 vs HT-2 is barely significant with P = 0.046).

4. Discussion

This re-analysis of a set of 20 MUNIX recordings from normal subjects has shown why this EMG measure is so strongly related to CMAP amplitude. Eq. (8), which is equivalent to the MUNIX derivation in the commercial Excel program, shows that the dependence of MUNIX on SIPs is limited to its dependence on the form factor G_{SIP} , at an arbitrary low level of voluntary activity, when the mean rectified EMG is 0.02 mV. And the relationship between G_{SIP} and the level of EMG activity is highly consistent across normal subjects. The fact that MUNIX depends on G_{SIP} , but not on SIP amplitude, explains the finding in Figs. 4B and 5B that changing the sizes of all recorded motor units, for the same CMAP size, has very little effect on MUNIX, or on MUSIX, although MUSIX was proposed as an index of motor unit size.

A fundamental flaw in the MUNIX analysis of SIPs is the implicit assumption that motor unit size information is retained when motor unit potentials overlap, whereas it is rapidly lost, and the form factor progressively approaches that for Gaussian noise (Fig. 1). When motor units extend their territories by collateral reinnervation, motor unit potentials may become big enough that the 0.02 mV level of rectified EMG is attained with rather little overlap of the potentials and $G_{SIP(20\mu V)}$ increases. In this situation the assumptions behind MUNIX become more reasonable, and MUNIX values become sensitive to SIP as well as CMAP amplitude. Only in exceptional cases, however, is it found that the selective doubling of motor unit potential amplitudes has the effect that it should, of halving MUNIX and doubling MUSIX. We conclude that MUNIX should not be used as a measure of the number of functional motor units in a muscle.

MUNIX is particularly misleading for muscles with normal or small motor units, which can only generate a mean (rectified) SIP level of 0.02 mV by superimposition of motor unit potentials. It was noticed early on that there is a strong correlation between MUNIX and CMAP amplitude in healthy subjects (e.g. Fig. 2 in Nandedkar et al., 2010), and the crucial dependence of MUNIX, as well as CMAP amplitude, on electrode displacement was stressed. It was apparently never appreciated, however, that in such subjects

(where $G_{SIP(20\mu V)}$ is always close to 1.42) MUNIX provides little or no useful information beyond that provided by CMAP amplitude. Similarly, we found that the apparent reproducibility of MUNIX values in healthy controls was almost entirely dependent on the reproducibility of CMAP amplitudes.

It is not only for healthy subjects that MUNIX is unduly dependent on CMAP amplitude. Table 1 shows that for ALS patients also, MUNIX and MUSIX are not nearly as sensitive to selective changes in SIP amplitudes as a true MUNE method would be. It might be argued that MUNIX never claimed to be a MUNE method, which is why the word 'index' rather than 'estimate' was used, and that what really matters is that MUNIX is sensitive to the lack of motor units in ALS and able to track the further loss of motor units with time (Neuwirth et al., 2015). However, our experience urges caution in accepting that MUNIX provides more information than CMAP amplitude for this purpose also. As mentioned in the Introduction, our previous paper found that two different MUNE methods correlated better with each other than with MUNIX or CMAP amplitude, whereas MUNIX values were most closely related to CMAP amplitude. Most important, perhaps, is that when we performed ROC analyses to determine how well the different methods could distinguish healthy controls from ALS patients, the areas under the curve fell into two groups: values for MScanFit MUNE (0.930) and MPS MUNE (0.899) were significantly higher than those for MUNIX and CMAP amplitude (both 0.831). In other words, unlike the two MUNE methods, MUNIX was no better than CMAP amplitude at determining whether an individual was likely to have ALS or not.

Another reason why MUNIX has found favour with many clinical neurophysiologists is because it is simpler and faster to record than conventional MUNE methods such as MPS. Thus in our study, MUNIX recordings took an average of 6.34 min, as against 13.24 min for MPS MUNE. However, the latest MUNIX guidelines recommend recording a minimum of 20 SIP epochs of 500 ms duration, rather than the 10 epochs of 300 ms duration in our study (Nandedkar, 2018). This would reduce the time advantage over MPS, and also make it slower than MScanFit MUNE, which took an average of 6.27 min per subject.

In conclusion, we have been disappointed in our findings with the MUNIX technique, which do not justify its common use as a MUNE method. In comparison with the MPS and MScanFit MUNE methods it is much too dependent on CMAP amplitude, and too

insensitive to motor unit potential amplitudes, to provide reliable information about motor unit numbers. The reasons for this are easy to see when the definition of MUNIX is expressed in terms of the form factor for surface EMG interference pattern (Eq. (8)) which varies rather little when motor unit potentials overlap. A simple test of the validity of MUNIX and MUSIX values is to simulate doubling motor unit size for the same size CMAP by changing the gain of the SIP recordings (Figs. 4B and 5B).

Declaration of Competing Interest

Professor Hugh Bostock receives royalties from UCL for sales of his Qtrac software used in this study. The other authors have no conflicts of interest to disclose.

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